

Infectious Diseases of the Upper Respiratory Tract: Implications for Toxicology Studies

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The consequences of adventitious infectious agents upon the interpretation of toxicology studies performed in rats and mice are incompletely understood. Several prevalent murine pathogens cause alterations of the respiratory system that can confuse the assessment of chemically induced airway injury. In some instances the pathogenesis of infection with these agents has been relatively well studied in the lower respiratory tract. However, there are few well-controlled studies that have examined the upper respiratory region, which result in interpretive problems for toxicologic pathologists. The conduct and interpretation of both short-term and chronic rodent bioassays can be compromised by both the clinical and subclinical manifestations of infectious diseases. This paper reviews several important infectious diseases of the upper airway of rats and mice and discusses the potential influence of these conditions on the results of toxicology studies.

Introduction

The validity and reproducibility of rodent toxicology studies depend, in part, on the interaction of numerous environmental, microbial, and genetic factors. Control of important animal and extrinsic factors forms the basis of laboratory animal science programs in toxicology. Despite numerous advances and sophistication in the field of laboratory animal medicine, adventitious murine microbial agents continue to pose a threat to both short- and long-term studies that use rats and mice.

Programs for prevention, detection, elimination, and control of rodent infectious agents are necessary in toxicology research and testing. These programs are of particular importance to both toxicologists and pathologists responsible for conducting and interpreting rodent bioassays. Specific procedures and equipment used in inhalation toxicity experiments can contribute significantly to the spread of disease and the interaction of infectious agents with inhaled toxicants. Many factors, such as inhalation caging; cage rotation schemes; inhalation chamber microenvironments; food and water deprivation during exposures; and stresses from crowding, frequent handling, and servicing of animals, all play important roles in the interplay between pathogen and chemical exposure.

The complications of rodent infectious agents to toxicology research and testing has been the subject of increasing awareness and review (1,2). Recent rodent serology surveys indicate that sialodacryoadenitis virus (SDAV), Sendai virus, and *Mycoplasma pulmonis* are three of the most prevalent murine pathogens (3). All three agents cause significant rodent respiratory disease, with lesions in the upper airways, including the nasal passages. In addition to being a common site for microbial-induced disease, the upper respiratory tract is a target organ of chemically induced injury. This paper will review the respiratory pathology of several important murine pathogens and discuss their importance in toxicology and carcinogenicity studies.

Viral Infection

Sialodacryoadenitis Virus

Sialodacryoadenitis virus is a common, highly infectious coronavirus affecting rats and causing a self-limiting necrosis and inflammation of mixed or serous salivary glands, lacrimal glands, and upper airway epithelium (4,5). Respiratory tract lesions are generally confined to the upper airway and usually precede the inflammatory changes that occur in the exocrine tissues of the head (6). Gross lesions consisting of edematous and/or inflammatory changes are sometimes noted in extrapulmonary tissue in mixed or serous salivary glands, exorbital lacrimal glands, Harderian glands, periglandular connective tissue, cervical lymph nodes, or the thymus.

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Microscopic lesions in experimental SDAV infection begin in the nasal respiratory epithelium approximately 48 hr postinfection. Initial respiratory epithelial necrosis accompanied by congestion and edema is rapidly followed by a mixed inflammatory cell infiltrate of the lamina propria. Necrosis primarily occurs in the respiratory epithelium lining the ventral turbinates and lateral wall of the nasal cavity but usually spares the olfactory mucosa. The nasal meatuses may be filled with exudate composed of necrotic epithelial cells admixed with inflammatory cells and mucus. Serous mucosal glands of the nasopharynx generally sustain relatively mild injury (6), although necrosis of ducts and acini does occur. Lesions similar to those in the nasopharynx, but milder and less uniform, are found in the trachea. The rhinotracheitis of SDAV is usually self-limiting and it is resolved by the end of the second week of infection (6). Although not as prevalent or as severe as upper respiratory tract lesions, microscopic changes do occur in the lower respiratory tract in experimental infections of rats (7), consisting of focal nonsuppurative bronchiolitis and peribronchial lymphocytic infiltration.

Although SDAV infection is generally a self-limiting disease without significant mortality, it can cause marked effects on toxicology studies through clinical disease or subclinical manifestations. Rats with SDAV infection often have reduced food consumption, weight loss, and reduced breeding performance — all of which can affect toxicology studies. The interactions of SDAV with other respiratory pathogens and chemical agents is not well established. Recent experimental evidence suggests that SDAV infection depletes salivary epidermal growth factor (EGF) and may thereby affect EGF-dependent cell growth processes and experimental carcinogenesis studies in rats (8).

Results of a 2-year inhalation toxicity study of methylene chloride were clouded by SDAV infection of rats early in the study (9). In this study, a low number of male rats exposed to the two highest doses of the chemical had an increased incidence of sarcomas in the ventral neck region. The relevance and toxicological significance of these neoplasms was questionable because the species and sex specificity of the response was inconsistent with the body of knowledge on the toxicity of the chemical. It was postulated that the tumor response may have been due to a combination of viral infection and exposure to high concentrations of methylene chloride. This case exemplifies the problems that may occur in toxicologic studies when unusual and unexpected lesions are found in animals that had infectious processes within the target organ of toxicity.

Sendai Virus

Sendai virus is a very common respiratory pathogen of laboratory rodents that has complicated numerous toxicology and carcinogenicity studies (10). In mice and rats, this paramyxovirus causes clinical and subclinical changes with great strain variability in disease expression and resultant pathologic lesions. The virus has a marked tropism for the respiratory tract, including the nasal cavity.

The pathogenesis of natural and experimental Sendai virus infection has been well described for mice (11–13), and recently the course of experimental infection in the rat has been reported (14). Sendai virus infection causes rhinitis, tracheobronchitis, bronchiolitis, and varying degrees of alveolitis in both rats and mice. There is marked strain variability in qualitative, quantitative, and chronological aspects of lesion development, which causes difficulty in making generalizations regarding the pathology of the respiratory tract. Although there are many excellent descriptive studies of the histogenesis of Sendai virus-induced lesions within the lower respiratory tract, few pathology reports include a description of lesions in the nasal cavity and upper airway.

Nasal lesions have been well described in experimental infections of the Sprague-Dawley rat (14). In this model, a rapid development of severe rhinitis with marked infiltration of the epithelium and lamina propria with lymphocytes and some neutrophils occurred at 1 day postinfection. Lesions were most severe in the respiratory epithelium of the naso- and maxilloturbinates, compared to minimal lesions in the olfactory epithelium of the ethmoid region. Over the next 4 days postinfection, there was a progressive accumulation of inflammatory cells, chiefly of lymphocytes, in the lamina propria. The lesions expanded to involve the middle portions of the nasal septum. Respiratory epithelial cells exhibited pyknosis and karyorrhexis, particularly in the more basilar regions of the mucosa. Exudate was noted in the ethmoid region, although there was little inflammation in that area. Between 5 and 21 days postinfection, the severity of the nasal epithelial necrosis, inflammatory cell infiltrate, and luminal exudate decreased. By day 28 postinfection, no discernable lesions were noted in experimentally infected rats.

There are numerous systemic effects (Table 1) that toxicologists and pathologists need to consider when interpreting the impact of Sendai virus infection upon study results (10,15). Studies can be compromised through reduction in animal numbers, changes in immune function, xenobiotic metabolism, and physiology (10). Sendai virus infection has altered the course of chemically induced pulmonary carcinogenesis in strain A mice by reducing the number of resultant lung tumors (16). In addition, the pulmonary carcinogenic responses have been altered in Sendai virus infected Balb/c mice following exposure to urethane (17).

Table 1. Systemic effects of Sendai virus infection.

Effect	Species
Depressed peripheral T-cell mitogenesis	Rat
Decreased severity of adjuvant arthritis	Rat
Increased activity of splenic NK cells	Mouse
Immune complexes in renal glomeruli	Mouse
Altered regulation of <i>in vitro</i> immune response to heterologous erythrocytes	Mouse
Decreased intrapulmonary killing of <i>Pasteurella pneumotropica</i>	Mouse
Phagocytic defects in pulmonary macrophages	Mouse

Mycoplasma Infection

Murine respiratory mycoplasmosis (MRM), due to *Mycoplasma pulmonis*, is a naturally occurring, slowly progressing, chronic disease in rats and mice. Numerous respiratory responses are associated with *M. pulmonis* infection of rodents (Table 2) (18). The prevalence of the pathogen and the numerous respiratory and systemic effects of infection make this disease one of the most important entities for pathologists and toxicologists during studies of the respiratory tract. Respiratory mycoplasmosis is often clinically silent, although lesions of the upper respiratory tract commonly occur (19).

The principal lesions of MRM in rats include rhinitis, otitis media, laryngitis, and tracheitis (20). Gross lesions in the upper respiratory tract are generally not discernable, although rats may occasionally show mucopurulent nasal exudate and/or porphyrin-tinted oculonasal discharge. The microscopic pathology of MRM is characterized by epithelial changes including cellular hypertrophy and hyperplasia, as well as metaplastic changes. Neutrophilic exudation and lymphoplasmacytic infiltrates are common throughout the upper airway. Subepithelial lymphoid accumulations can occur in MRM-associated rhinitis (Plates 1 and 2). Ciliary loss and epithelial hyperplasias can be severe and quite extensive.

The relative importance of direct mycoplasmal damage, as opposed to immune and nonimmune inflammatory reactions for *M. pulmonis*, has yet to be completely discerned (19). It is known that cytolysis follows attachment of *M. pulmonis* to upper airway epithelial cells. Ciliastasis, loss of cilia, distension of intercellular spaces, cytoplasmic vacuolization, disruption of mitochondria, epithelial hyperplasia and metaplasia, and syncytial cell formation have also resulted following attachment of this organism (19).

The tympanic cavity of the ear may be completely filled with a neutrophilic exudate. Frequently in MRM-induced otitis media, the lining epithelium is hyperplastic and the luminal cavity may become filled with collagenous connective tissue. Thickened connective tissue lining the tympanic cavity may remain as a chronic sequellum of the infection. Epithelial hyperplasias and lymphoid cell accumulations are commonly found in the

larynx and trachea during MRM infections. The associated submucosal glands can become dilated with accumulation of mucopurulent debris.

Certain chemical agents, including ammonia, which is commonly found in the cage environment from soiled bedding, can exacerbate MRM in rats. Inhalation of the important industrial compound hexamethylphosphoramide (HMPA) can cause a synergistic enhancement of the progression and severity of MRM (21,22). In HMPA toxicity studies, nasal tumors, rhinitis, nasal epithelial degeneration, metaplasia, and dysplasia were noted in conjunction with an enhanced mortality from chronic pneumonia in infected rats. The increased mortality was possibly due to a chemically induced destruction of the mucociliary apparatus in the upper airway, which may have contributed to fatal lower respiratory infections. A chronic inhalation bioassay of propylene oxide revealed dose-dependent nasal epithelial proliferative lesions and two nasal adenomas in rats with intercurrent MRM (23). The significance of the proliferative nasal lesions, which appeared to be treatment related, was difficult to interpret, since their development may have been influenced by intercurrent infectious inflammatory disease.

Bacterial Infections

Although numerous bacteria can infect the upper airway of the rat and mouse, they are not generally prevalent in well-conducted toxicology studies begun with animals free of adventitious murine pathogens and maintained with modern methods of laboratory animal husbandry. However, the cilia-associated respiratory (CAR) bacillus has recently received attention. This gram-negative, filamentous, rod-shaped bacillus (24,25) infects both rats and mice and causes lesions morphologically similar to those of *M. pulmonis* infections. The CAR bacillus was generally overlooked in the past because it often occurred as a dual infection with *M. pulmonis*.

Lesions typical of mycoplasmal chronic respiratory disease have been noted following natural and experimental infection with the CAR bacillus. These lesions include massive accumulations of predominantly mononuclear inflammatory cells surrounding bronchi and bronchioles (Plate 3), as well as various degrees of suppurative bronchopneumonia, squamous metaplasia, and bronchiectasis. The ciliated epithelium of the airway is usually heavily colonized with filamentous bacteria, which can be readily demonstrated with Warthin-Starry silver impregnation techniques (Plate 4).

Little is known regarding the prevalence of this organism as a subclinical infection. Although lesions induced by the CAR bacillus have been described in the lungs of both rats and mice, there have been no descriptions of pathology in the upper airway where, presumably, the organism can also grow. Elucidation of the significance of this pathogen for rodent toxicity awaits further studies and characterization.

Table 2. Respiratory tract responses associated with *M. pulmonis* infection.

Airway epithelial cells
Hypertrophy and hyperplasia
Necrosis
Syncytial cell formation
Squamous metaplasia
Mucous cell hyperplasia
Hyperplasia Type II epithelial cells
Altered tumor response following carcinogen
Decreased mucociliary function
Altered surfactant
Changes in airway cytokinetics
Alterations in pulmonary lymphocytes

Fungal Infections

Descriptions of naturally occurring fungal infections of the respiratory tract of rodents are extremely rare. The histological features of fungal rhinitis, which occurred in high incidence in two separate chronic carcinogenicity studies in male Wistar rats (129/597 animals), have been described (26). Since these animals had titers to both Sendai and SDAV viruses, the authors suggested that viral-induced inflammation of the upper respiratory mucosa rendered the epithelium susceptible to the fungal infection. In addition to the 129 animals with fungal infection, 80 animals had suppurative rhinitis without evident fungal growth. An etiologic diagnosis of *Aspergillus fumigatus* was based upon characteristic morphology of the fruiting heads found in a small percentage of cases.

Aspergillus rhinitis was generally noninvasive and limited to the naso- and maxilloturbinates. Although purulent inflammation was noted in the olfactory epithelium of some cases, fungal growth was rarely noted in this region. Hyphal conglomerates were usually associated with foreign bodies (hair and plant material) and were surrounded by polymorphonuclear leukocytes, bacteria, debris, and nasal secretions. The underlying respiratory epithelium was usually hyperplastic, hypertrophic, or revealed squamous metaplasia. Subepithelial connective tissue and submucosal glands were often infiltrated by aggregates of lymphocytes, plasma cells, and neutrophils. A relatively small number of cases had epithelial necrosis associated with fungal invasion.

A review of cases of fungal rhinitis in the National Toxicology Program (NTP) archives revealed the occurrence of fungal rhinitis in eight bioassays. In the affected studies that encompassed animals that were both serologically negative as well as some bearing viral titers, cases occurred in both sexes of Fischer 344 rats in relatively low incidence (up to 10% of a dose group). A few scattered cases were noted in B6C3F₁ mice. The histologic appearance (Plates 5 and 6) was identical to that described in the literature in the Wistar rat. In the affected bioassays the chemical was administered by inhalation, feed, or gavage in several different laboratories. Foreign material was noted in most of the cases suggesting that particles from food or elsewhere may serve as local irritants and carriers for the fungus.

Discussion

One of the principal ways in which infectious diseases complicate toxicologic studies is by interference with the interpretation of the pathology data. In addition to alterations in the morphologic appearance of target organs, there can be changes in clinical signs of toxicity, food consumption, body weight gain, hematology, urinalysis, and serum chemistry parameters (27). Body weight gain depression of greater than 10% is often considered to be a sign of toxicity in treated groups of animals. The combined effects of infection and treatment influence food consumption and body weight gain to a greater extent

than infection or chemical treatment alone. This synergism can confound data interpretation (27).

It has recently become apparent that infectious agents can markedly affect the metabolism of foreign compounds by impairing hepatic mixed-function monooxygenases (cytochrome P-450-dependent monooxygenase system) (28). Alteration of xenobiotic metabolism has important ramifications for toxicologists who administer compounds that are subject to bioactivation and biotransformation. The mechanistic basis for the microbial-induced inhibition of biotransformation has not yet been completely elucidated. It appears that interferon induction as a result of infection can act as a mediator in depressing P-450, and it may have a direct action on the hemoprotein itself (28). Many of the important murine viral agents including Sendai virus are potent inducers of interferon (29). Infectious diseases can also modulate hepatic cytochrome P-450 by effects on the reticuloendothelial system. Furthermore, microbial agents that perturb Kupffer cells decrease drug biotransformation activity (28).

Effects on nonhepatic xenobiotic metabolism by infectious agents have received little attention. Several upper respiratory viral infections in man, including rhinoviruses and influenza viruses, are known to impair drug biotransformations by affecting the cytochrome P-450 system (28). Similar viral effects have been demonstrated in PR8 influenza-infected mice (30). Mice infected with this virus have significantly decreased pulmonary benzo[*a*]pyrene hydroxylase activity.

Nasal cytochrome P-450-dependent monooxygenases may be extremely important in the bioactivation and biotransformation of inhaled xenobiotics. In most species, the nasal olfactory concentration of these enzymes is second only to that found in the liver (31). Mouse hepatitis virus, a common mouse coronavirus infection, is reported to depress hepatic P-450 levels (32). Strains of this pathogen cause nasal cavity infections with necrotizing lesions in the olfactory mucosa (33). It is therefore possible that extrahepatic alterations of P-450 may result and be of importance to toxicology studies.

In addition to alterations of xenobiotic metabolism, infectious diseases cause other cellular effects that can modulate toxic and carcinogenic responses. The association between enhanced cell replication and cancer induction has gained the interests of many toxicologists (34). Gross and co-workers found that sites of cell replication correlated well with sites of tumor induction in the nasal cavities from rats exposed to formaldehyde in acute and chronic inhalation studies (35). Although it is uncertain how enhanced cell replication affects the carcinogenic process, such correlations suggest that a cause and effect relationship may exist. Infectious diseases that cause epithelial necrosis and repair lead to significant alterations in cell turnover. Microbial agents such as *M. pulmonis* are known to cause significant alterations in cell cycle kinetics in populations of upper airway epithelial cells (36). This clearly has profound implications for carcinogenesis studies in which cell turnover

may be an important contributing factor in the multistage process. Not only would the microbial infection and degree of cell turnover be important, but the chronology of the infection with regard to the chemical administration could prove critical.

Summary

A variety of important microbial pathogens including viruses, mycoplasmas, bacteria, and fungi infect the upper respiratory tract of the mouse and rat and result in significant pathologic alterations. The rodent upper respiratory tract may also be an important target organ in chronic bioassays. Toxicologists and pathologists need to understand the etiopathogenesis of common pathogen-related diseases in the murine respiratory tract, so that the effects of natural disease processes may be separated from chemically induced injury. Additional studies are needed to assess the impact of several of the more prevalent adventitious pathogens on the results of rodent bioassays.

The examination of the rodent nasal cavity has been overlooked in many previous experimental models of murine infectious disease. Descriptive pathology studies, which carefully examine the histogenesis of upper airway injury, are warranted for many of the common pathogens that complicate toxicology studies. In addition, the effects of murine infectious diseases on xenobiotic metabolism and cytokinetics in the upper airway should be further investigated.

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PLATE 1. Photomicrograph of the nasal septum from a rat with massive accumulation of mixed mononuclear inflammatory cells within the lamina propria. H&E, $\times 140$.

PLATE 2. Higher magnification of nasal septal region depicted in Plate 1. Dilation of glandular lumina with mononuclear and admixed polymorphonuclear leukocytes are present (arrow). The overlying respiratory mucosa appears normal. H&E, $\times 280$.

PLATE 3. Photomicrograph of lung section from mouse with CAR bacillus infection. Marked peribronchial inflammatory infiltrates distort the normal pulmonary architecture. H&E, $\times 60$.

PLATE 4. High power photomicrograph of the bronchial epithelium of a mouse with CAR bacillus infection. The dark staining material that lines the luminal cavity (arrow) represents the filamentous organisms. Warthin-Starry, $\times 780$.

PLATE 5. Photomicrograph of the nasal cavity from a F344 rat with *Aspergillus* rhinitis. The respiratory epithelium has early squamous metaplasia. A diffuse inflammatory infiltrate is present in the underlying lamina propria. The nasal cavity is filled with debris and polymorphonuclear leukocytes. H&E, $\times 280$.

PLATE 6. Fungal hyphae within nasal cavity debris of F344 rat with *Aspergillus* rhinitis. Gomori-methenamine silver, $\times 780$.